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FORMULATION AND CHARACTERIZATION OF NANOPARTICLES OF ATORVASTATIN CALCIUM

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Abstarct:

The present research work was a novel formulation by applying Vitamin E TPGS as an emulsifier to fabricate Nanoparticles by solvent dispersion/ nano precipitation for controlled release of Atorvastatin Investigation of the preparation, characterization and in-vitro release of the Nanoparticles was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimized. Our results demonstrated that vitamin E TPGS could be an efficient emulsifier for fabrication of polymeric nanoparticles, which can achieve excellent effects in drug encapsulation efficiency, size and size distribution and in vitro release kinetics of the nanoparticles. In this research, a drug encapsulation efficiency as high as 96% has been achieved. The particle size and size distribution strongly depends on the amount of TPGS added in the fabrication.Drug release kinetics indicated that drug release was best explained by Higuchi's equation, as these plots showed the highest linearity (r^2=0.978) but a close relationship was also noted with Zero order kinetics (r^2 =0.952)

Key Words: *Atorvastatin, Nanoparticles, solvent dispersion, drug encapsulation efficiency.*

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INTRODUCTION:

The prefix "nano" comes from the ancient Greek vavoc through the Latin nanus meaning very small. Nanotechnology defined as design characterization, production and applications of structures, devices and systems by controlling shape and size at nanometer scale. Nanoparticles (NPs) are defined as particulate dispersions or solid particles drug carrier that may or may not be biodegradable. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix.[1,2]

Atorvastatin calcium (AC) is a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor approved for clinical use as a lipid lowering agent. AC is belong to the BCS class second which having low solubility and high permeability. AC is the world's best selling drug is associated with poor oral bioavailability and serious adverse effects like rhabdomyolysis on chronic administration. A biodegradable nanoparticulate approach was introduced here with a view to improving the efficacy and safety of AC. Nanoparticles represent a promising drug delivery system of controlled and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue.[3,4]

MATERIALS AND METHOD:

Atorvastatin, Gift sample from Flamingo Pharmaceutical at Nanded, PLGA, TPGS, Acetone, Dialysis membrane Research Lab Fine Chem Industries, Mumbai.

Solvent dispersion (Nanoprecipitation)

The nanoparticles are prepared by dissolving the drug in organic phase along with the polymer (PLGA) and added to the aqueous solution containing TPGS which acts as an emulsifier. The solution of organic phase was added in drop wise into aqueous phase under homogenization at 11,000 rpm. The dispersion was kept under magnetic stirring for 4hrs at room temperature. The solution is kept under reduced pressure for about 2-3min. This process forms nanoparticles loaded with drug.

Table 1: Composition of the Nanoparticles

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
PLGA (50:50)(mg)	13	13	13	25	50	75	100	125
TPGS(%g/ml)	0.015	0.03	0.06	0.03	0.03	0.03	0.03	0.03
Atorvastatin (mg)	5	5	5	5	5	5	5	5
Acetone (ml)	3	3	3	3	3	3	3	3
Water (ml)	10	10	10	10	10	10	10	10

Note: In above all formulations (F1 to F8) 5mg of the drug was added instead of original dose of the API (20mg). The above formulations were prepared and the entrapment efficiency was determined for choosing best formulation.

EVALUATION OF ATORVASTATIN LOADED NANOPARTICLES

1. Organolaptic properties
2. Particle size
3. Zeta potential
4. Entrapment efficiency
5. In vitro drug release
6. Stability Study

Organolaptic properties

In which the colour, odour, & appearance are detected.

Melting point

Melting point of Atorvastatin is determined by using Thieles tube method.

Determination of λmax of Atorvastatin

Particle size and zeta potential

The particle size and zeta potential of nanoparticles were measured by photon correlation spectroscopy using a Zetasizer 3000 HSA (Malvern, UK). Samples were diluted appropriately with the aqueous phase of the formulation. Zeta potential measurements were carried out at 25° degree C. Zeta Potential is an important tool for prediction of long term stability and understanding the state of the nanoparticle surface.

Lyophilization

The obtained centrifuged samples were lyophilized and stored at 2-8^{0}C. The samples are lyophilized to attain stability. The obtained lyophilized powder is

utilized for determination of entrapment efficiency and in-vitro drug release parameters.

Drug encapsulation efficiency

Lyophilized nanoparticles 3mg were dissolved in 1ml of diluents and the drug amount was determined by HPLC analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Atorvastatin in nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Atorvastatin PLGA nanoparticles was expressed as loading capacity.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro atorvastatin release

10 mg drug equivalent freeze dried Atorvastatin loaded nanoparticles were dispersed in 3 ml pH 7.4 phosphate buffer solution which is transferred in dialysis bag and suspended in 100 ml of isotonic pH 7.4 Phosphate buffer solution (PBS). The bag was placed under magnetic stirring in a water bath maintained at 37 ± 0.5° C. At fixed time intervals 5ml of samples were taken out and fresh buffer was replaced. The obtained solution was analyzed by HPLC to determine the drug content.

Mathematical modeling of the drug release

The controlled release of drugs can be achieved by incorporating solutes, either in dissolved or in dispersed form, in polymers. During the design stage of these formulations, it is desirable to develop and use simple yet sophisticated mathematical models to describe release kinetics. From a mathematical modeling point of view, controlled-release systems can be classified according to the physical mechanisms of the release of the incorporated solute. Mathematical modeling of the release kinetics of specific classes of controlled-release systems may be used to predict solute release rates from and solute diffusion behavior through polymers and to elucidate the physical mechanisms of solute transport by simply comparing the release data to mathematical models. Diffusion-controlled systems contain a reservoir, matrix, and porous membrane. Mathematical models exist for diffusion systems in which solute release from the matrix is also important. The mechanism of drug release from the formulations during the diffusion in pH 7.4 phosphate buffer was determined using the Zero order, First order, Higuchi equation and Korsmeyer Peppas plot.

Zero order equation

This equation describes the systems where the release rate is independent of the concentration of the dissolved species. The dissolution data are fitted into the zero order equation:

$$\mathbf{Q = Q_0 . K_0 t}$$

Where,

Q = Amount of drug released at time t.

Q_0 = Amount of drug released initially.

K_0 = Zero order rate constant.

A graph of concentration vs. time would yield a straight line with a slope equal to K_0 and the intercept at the origin of the axes. The zero order plot is derived from plotting the cumulative percent drug dissolved vs time.

First order equation

The first order equation describes the release from systems where the dissolution rate is dependent upon the concentration of the dissolving species.

Release behavior generally follows the following first order release equation:

$$\mathbf{\ln M = \ln M_0 - K_1 t}$$

Where, M is the amount of drug un-dissolved at time t,

M0 is the amount of drug un-dissolved at t = 0 and

K_1 is the corresponding release rate constant.

A graph of log concentration of drug remaining Vs time yields a straight line with a negative slope.

higuchi square root law

A form of the Higuchi Square Root Law is given by equation:

$$\mathbf{Q = K_H \sqrt{t}}$$

Where, Q = Amount of drug dissolved at time t

K_H = Higuchi rate constant

The Higuchi square root equation describes the release from systems where the solid drug is dispersed in an insoluble matrix, and the rate of drug release is related to the rate of drug diffusion.

korsemeyer peppas equation

The Korsmeyer's equation which derived from the linear line of log cumulative percentage drug release Vs log time curve is

$$\mathbf{M_t / M_\infty = K t^n}$$

Where Mt and M∞ are the absolute and the cumulative amount of drug released in time t and infinite time; k is a constant incorporating the structural and geometric characteristics of the device and n is the release exponent which is indicative of the mechanism of release. This is also known as the power law.

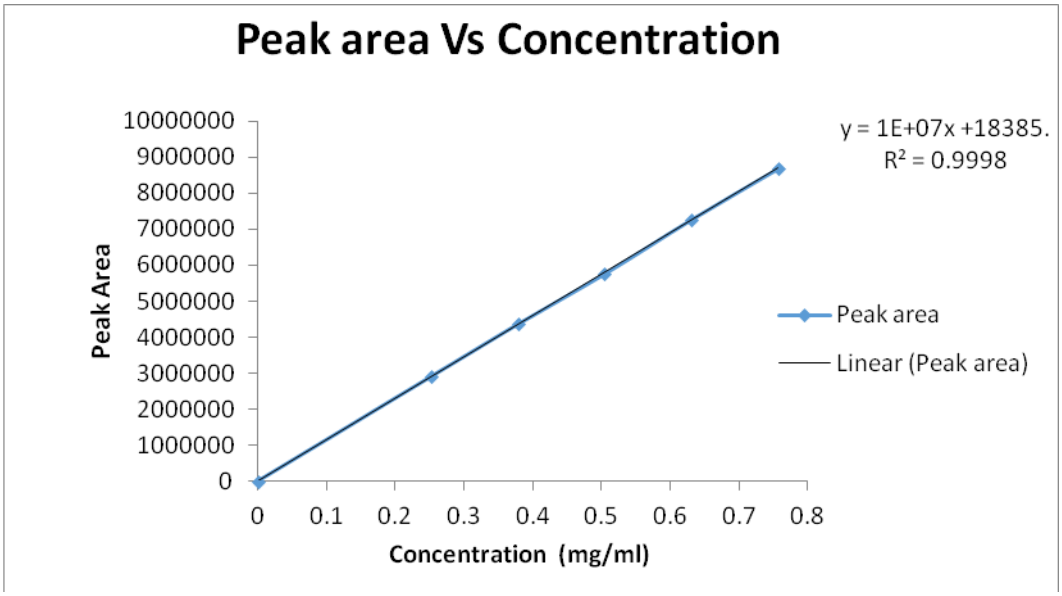
RESULT AND DISCUSSION:

HPLC METHOD

Samples collected in diffusion studies were analyzed by HPLC technique. For this purpose a standard plot was plotted in HPLC by using reference standard of Atorvastatin

Table 2: Standard Curve of Atorvastatin:

S.No	Concentration (mg/ml)	Peak area
1	0	0
2	0.2525	2912222
3	0.37875	4364555
4	0.505	5760005
5	0.6312	7265583
6	0.7575	8682663



Organoleptic property: Colour of Atorvastatin is White, and its Odour less. Atorvastatin is amorphous in nature.
Melting Point: The melting point of Atorvastatin found to be 158-160 ℃
λmax determination: The λmax of Atorvastatin in methanol solution was found to be 246 nm.

Table 3: Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency, Particle size, Zeta Potential and Drug Loading

Batch No	Particle size (nm)	Zeta potential (mV)	Drug Loaded (mg)	Entrapment Efficiency (%)
F1	251.2	-0.263	0.3	5
F2	151.5	-5.14	0.27	5.2
F3	532.5	-1.89	0.22	4.7
F4	---	---	1.10	28
F5	106.9	-23.09	1.8	36
F6	133.2	-24.8	3.29	63
F7	156.2	-24.3	4.23	80
F8	121.2	-26.9	4.6	94

The first part of the plan of work was to optimize the concentration of surfactant to be used in the formulation of nanoparticles. To achieve this, the first three formulations were planned with TPGS concentrations 0.015%, 0.03% and 0.06% respectively. The optimization of surfactant concentration was done on the basis of particle size and entrapment efficiency of nanoparticles obtained.

As the least particle size and best entrapment efficiency was obtained for F2 formulation when compared to F1 and F3, it was decided that the 0.03% of TPGS was the optimum concentration to be used in further formulations.

The next part of the plan of work was to optimize the drug polymer ratio. For this, 5 batches were planned (F4 to F8) using the drug polymer ratios of 1:5, 1:10, 1:15, 1:20 and 1:25 respectively. The optimum drug polymer ratio was selected on the basis of entrapment efficiency of the polymer. The entrapment efficiency was found to be very low for 1:5 (21%) and 1:10 (38%) drug polymer ratio. In case of F6, F7 and F8 formulations the entrapment efficiencies were found to be 63%, 80% and 94% respectively. It indicated that there was no further increase in entrapment efficiency even when the polymer concentration was increased. After that we perform the invitro diffusion study for all 8 batches.

Table 4: Formulations used for in vitro diffusion study

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
PLGA	13	13	13	25	50	75	100	125
TPGS%(g/ml)	0.015	0.03	0.06	0.03	0.03	0.03	0.03	0.03
Atorvastatin (mg)	5	5	5	5	5	5	5	5
Acetone (ml)	3	3	3	3	3	3	3	3
Water (ml)	10	10	10	10	10	10	10	10

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 10 hours. Initially the release of drug from all the three batches was found to be about 25-35% in 1 hours. This was due to the release of adsorbed drug from the surface of Nanoparticles. Later on a constant and slow drug release was observed for 10hrs.

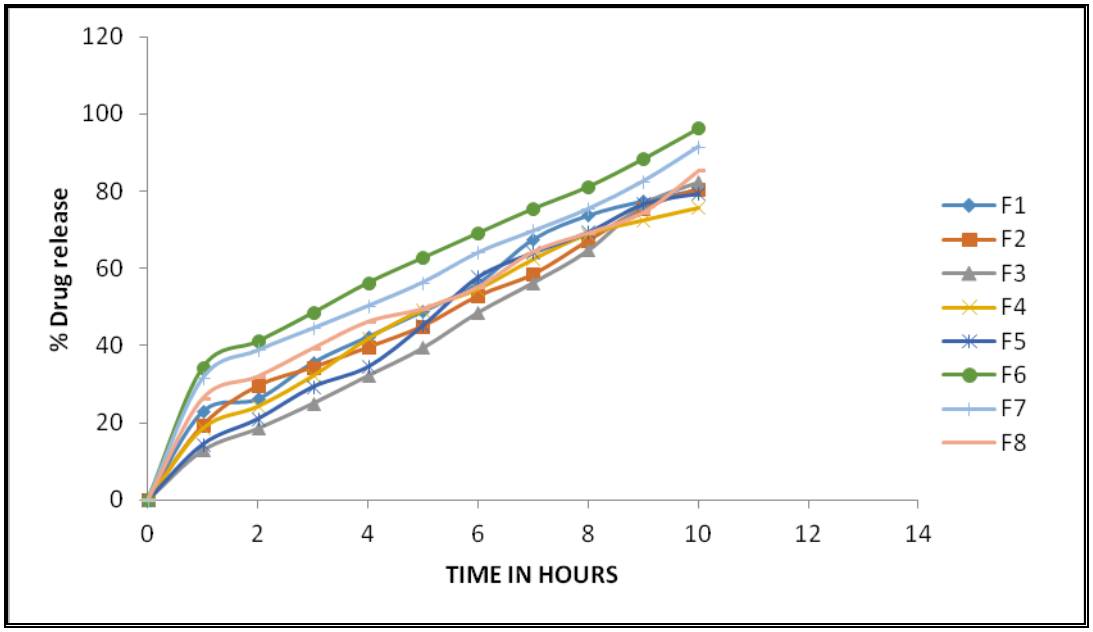


Figure 2: In vitro diffusion study of batches F1 to F8

The drug diffusion for F6, F7 and F8 formulations was found to be approximately same i.e., 96.4%, 91.5% and 85.4% respectively. Therefore the F8 formulation which had drug polymer ratio of 1:25 was decided to be the optimized formulation.

Optimized formulations:

Based on the entrapment efficiency, a set of formulations (F6, F7 and F8) were considered as optimized compositions.

Diffusion study profile for F6, F7 and F8 formulations

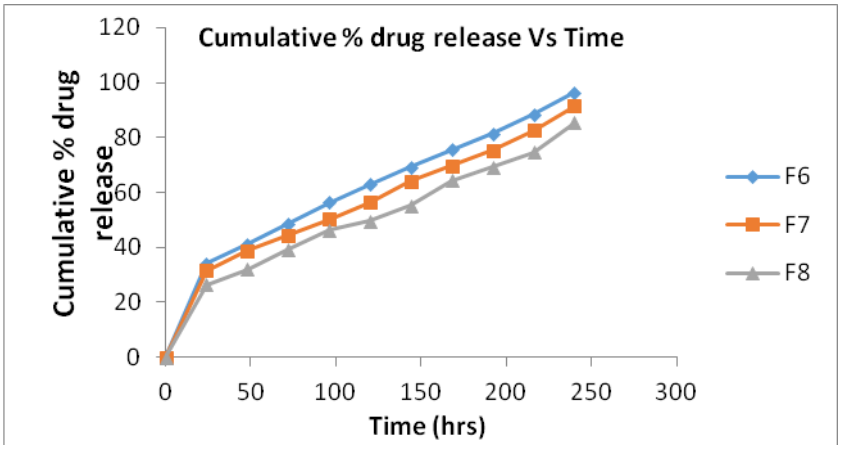


Fig 3: Diffusion study profile Cumulative % release Vs Time (hrs)

Zero order plot for F8 formulation

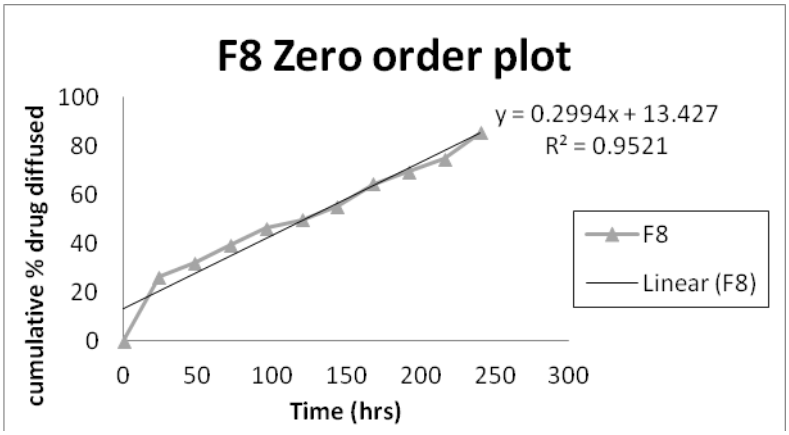


Fig 4: Zero order plot For F8 Formulation

The drug release from the Nanoparticles was found to follow Zero order release based on the "r" value obtained for Zero order (0.952) and first order (0.935) for F8 formulation. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.978 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to

be of Anomalous diffusion mechanism based on the "n" value of 0.774 obtained for Peppa's equation.

CONCLUSION:

Atorvastatin calcium (AC) is a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor approved for clinical use as a lipid lowering agent. AC is belong to the BCS class second which having low solubility and high permeability.The present research proposed a novel formulation by applying TPGS as an emulsifier to fabricate Nanoparticles by solvent dispersion/ nanoprecipitation for controlled release of Atorvastatin Investigation of the preparation, characterization and in-vitro release of the Nanoparticles was carried out. The different formulations of with various ratios of drug-polymer and surfactant were evaluated and optimized. Our results demonstrated that vitamin E TPGS could be an efficient emulsifier for fabrication of polymeric

nanoparticles, which can achieve excellent effects in drug encapsulation efficiency, size and size distribution and in vitro release kinetics of the nanoparticles.

In this research, a drug encapsulation efficiency as high as 94% has been achieved. The particle size and size distribution strongly depends on the amount of TPGS added in the fabrication. Optimized formulation of nanoparticle show the better drug release.

Drug release kinetics indicated that drug release was best explained by Higuchi's equation, as these plots showed the highest linearity (r^2=0.978) but a close relationship was also noted with Zero order kinetics (r^2 =0.952). Thus the objective of present work was achieved successfully.

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